VIRTUAL SCREENING AND ADMET ANALYSIS OF ENDOPEPTIDASE INHIBITORS

Kumar R[1], Patel K[3], Cai S[3] and Singh BR[1,2]
[1]*Botulinum Research Center, Institute of Advanced Sciences, Dartmouth, MA 02747*
[2]*Prime Bio, Inc., Dartmouth, MA 02747*
[3]*Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, North Dartmouth, MA 02747*

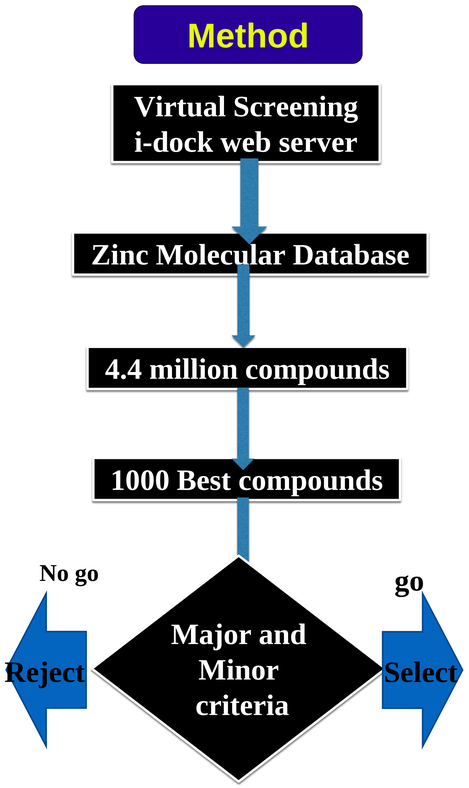
Abstract

Botulinum neurotoxin is causative agent of the life-threatening disease botulism. Several therapeutic approaches have been tried previously. Various studies, including antibody treatments, vaccine development, receptor decoy, and small molecule candidates, have been used to develop possible candidates as inhibitors of the endopeptidase activity of BoNT/A.. Conventional approaches are not very successful in designing the effective inhibitor candidates against BoNT (Botulinum Neurotoxin). Although these approaches investigated several family of compounds, such as hydroxamates and quinoline, but they fail to address the structural requirements needed in small molecules for effective inhibition. The challenges lie in translating the result from *in vitro* to *ex vivo/in vivo* assays and finally to clinical trials. Conventional approaches to design an effective inhibitor for BoNT endopeptidase activity have not succeeded so far. In this work, we used random selection approach to virtually screen ~ 4.4 million compounds. We determined *in silico* binding potential to BoNT/A light chain using predefined criteria, and performed *in silico* ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction of those compounds.

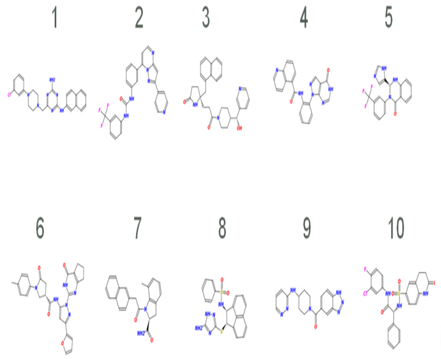
Unique Properties of Botulinum Neurotoxin Endopeptidase

1. Zn-mettalloprotease family ---- Zn-binding motif HEXXH+E.
2. a/b globular protein.
3. Recognize tertiary structure of the substrate.
4. Cleavage site is specific site out of several identical peptide bonds present in their respective target protein.
5. Two exosites : a and b.
6. Loops 50/60, 60/70, 170, 250 and 370 play major roles in recognition and activity.
7. Exceptionally high stability inside the cell.
8. Active molten globule.
9. Flexible active site. and negatively charged.
10. Active site crevice is very deep (20 A)
11. Large enzyme-substrate interface 4840 A^2
12. Substrate should mimic active site environment, in other way, it should have balance of hydrophobicity and polarity.

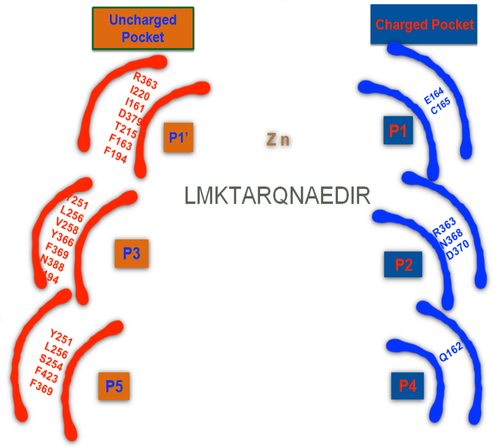
Method



Selected Molecules



Structural requirements



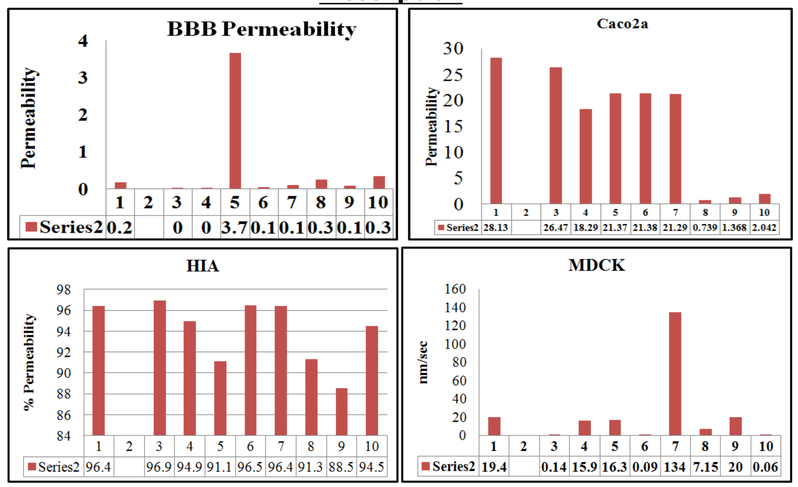
Major criteria

Molecule should have H- bond with active site residues HIS223, HIS227, GLU224, GLU262
H- bond or pi – pi Interaction or both with Arg363
Either H-bond or hyrophobic or pi-pi interactions with PHE163 or PHE193.

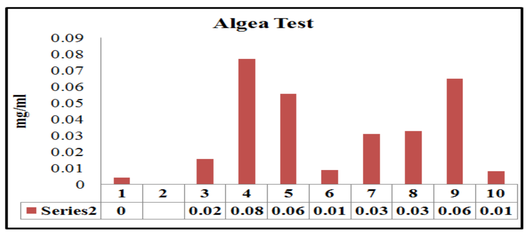
Minor Criteria

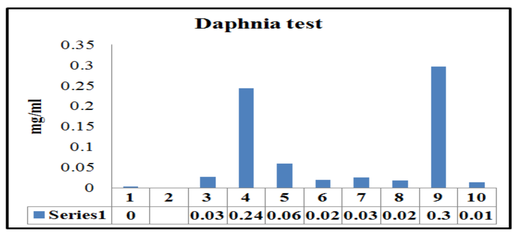
Any kind of interactions with PHE TYR366, VAL70, ASP370, GLU162, THR215, THR220 or PHE369

Absorption



Toxicity





Compound No.	Ames Test	Carcino Mouse	Carcino Rat	hERG_inhibition
1	Mutagen	positive	Negative	high_risk
2				
3	mutagen	negative	negative	medium_risk
4	mutagen	negative	positive	high_risk
5	mutagen	negative	positive	medium_risk
6	mutagen	negative	positive	medium_risk
7	mutagen	negative	negative	high_risk
8	non-mutagen	positive	negative	ambiguous
9	mutagen	negative	negative	medium_risk
10	non-mutagen	positive	negative	ambiguous

Drugability (no. of violations per rule)

Compound No.	CMC like	Leadlike Rule violation	MDDR rule violations	Lipinki rule of five violation	WDI like rule violation
1	1	2	1	0	0
2					
3	1	2	0	0	8
4	0	1	1	0	0
5	0	2	1	0	0
6	2	1	1	0	4
7	0	1	1	0	0
8	0	2	1	0	0
9	0	0	1	0	0
10	1	2	1	0	3

Conclusions

1. In this work, we performed adsorption, toxicity and drugability characteristics of selected compounds, from virtual screening.
2. Out of 10 compounds, compound 7 has better absorption property with low per meability to BBB.
3. Carcinogenicity of compound 7 is low. However, Daphnia toxicity is high (less concentration of the compound is toxic to Daphnia), hERG inhibition and plas ma protein binding could be an issue. But proper SAR can improve this compo und.

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